

In the Claims

The following listing of claims replaces all prior listings and versions of claim for this application.

1. (Previously Amended) A retroviral-infected immortalized human keratinocyte or melanocyte cell line prepared by infection of human keratinocytes or melanocytes with a retroviral construct that immortalizes cells, which immortalized human keratinocyte or melanocyte cell line retains the ability to differentiate and to express proteins and enzymes which are expressed by normal differentiated keratinocytes or melanocytes even after high passage in tissue culture, wherein the keratinocyte cell line expresses keratin proteins expressed by normal differentiated keratinocytes and the melanocyte cell line expresses melanin proteins expressed by normal differentiated melanocytes.

2. (Previously Amended) The retroviral-infected immortalized keratinocyte cell line according to claim 1, wherein said cell line expresses other proteins which are expressed by normal differentiated keratinocytes.

3. (Previously Amended) The retroviral-infected immortalized keratinocyte cell line according to claim 2, wherein said keratin proteins include Keratin K1/10, Keratin K14 and said other proteins include involucrin, filaggrin and loricrin.

4. (Previously Amended) The retroviral-infected immortalized keratinocyte cell line according to claim 1, wherein the cell line exhibits a CYP450 profile which is identical or substantially identical to that of normal differentiated keratinocytes.

5. (Previously Amended) The retroviral-infected An immortalized keratinocyte cell line according to claim 4, wherein the cell line expresses CYP450 1A1, 2C, 2E1 and 3A5 but does not express CYP450 1A2, 2A6, 2B6 and 2D6.

6. (Previously Amended) The retroviral-infected immortalized human keratinocyte cell line according to claim 1, which is produced under serum-free conditions without the use of feeder cells.

7. (Previously Amended) The retroviral-infected immortalized human melanocyte cell line according to claim 1, which is produced under serum-free conditions without the use of feeder cells.

8. (Original) An SV40 T-Antigen immortalized human skin cell line selected from the group consisting of the keratinocyte lines DK2-NR (DSM ACC2238), DK3-NR (DSM ACC2239) and FK2-NR (DSM ACC2240) and the melanocyte line DM2-NR (CNCM I-1796).

9. (Previously Amended) The retroviral-infected immortalized keratinocyte or melanocyte cell line according to claim 1, which expresses mRNA encoding glutathione-S-transferase GST- π , GST- μ . and GST- α .

10. (Previously Amended) The retroviral-infected immortalized keratinocyte cell line according to claim 1, which when cultured in organotypic culture forms a highly stratified and polarized epithelium having cornified superficial layers in the absence of serum or feeder cells.

11. (Previously Amended) The retroviral-infected immortalized keratinocyte cell line according to claim 1, which expresses collagenase type 1 and TNF- α when treated with phorbol esters.

12. (Previously Amended) A retroviral-infected immortalized human keratinocyte or melanocyte cell line that retains the ability to differentiate and to express proteins and enzymes which are expressed by normal differentiated keratinocytes or melanocytes even after high passage in tissue culture, wherein the cell line is prepared by:

- (i) obtaining a human skin tissue sample;
- (ii) preparing said skin sample for culturing *in vitro*;
- (iii) obtaining keratinocytes and/or melanocytes from said prepared skin sample and seeding said keratinocytes and/or melanocytes into a serum-free growth medium, onto culture plates provided with a coating comprising fibronectin, type 1 collagen and BSA which facilitates cell attachment and growth;
- (iv) changing the medium as necessary to optimize confluent growth of the cultured cells while continuously maintaining the coating on the culture plates;
- (v) transferring the keratinocytes or melanocytes into a serum-free selection medium onto similarly precoated culture plates;
- (vi) infecting the keratinocytes or melanocytes with a retroviral construct that immortalizes cells;

(vii) transferring the resultant immortalized keratinocytes or melanocytes to a serum-free proliferation medium suitable for proliferating immortalized keratinocytes or melanocytes onto similarly precoated culture plates; and

(viii) transferring the resultant proliferated keratinocytes to a serum-free differentiation medium which contains high calcium onto similarly precoated culture dishes.

13. (Original) The cell line according to claim 12 further comprising at least one of the following:

the retroviral construct is the SV40 construct pLXSHD+SV40(#328) or the HPV16 construct pLXSHD+E6/E7;

the serum-free medium in step (iii) is NR-3 medium;

the medium in step (v) is the NR-3 medium or the NR-4 medium;

the proliferation medium in step (vii) is the NR-2 medium or the NR-3 medium and M2 medium for melanocytes; and

the differentiation medium in step (viii) is the NR-2 medium or modified MCDB 153 medium which contains a calcium content of at least 1.5 mM.

14-19. (Cancelled)

20. (Previously Presented) The retroviral-infected immortalized human melanocyte cell line according to claim 1 wherein the cell line expresses vimentin, which is expressed by normal differentiated melanocyte cell line.